IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Inose, et al.
Appl. No.	:	10/553,376
Filed	:	October 19, 2005
For	:	METHOD FOR ISOLATING NUCLEIC ACIDS, AND A KIT AND APPARATUS FOR NUCLEIC ACID ISOLATION

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
Alexandria, VA 22313-1450

Dear Sir:

I, Satoshi Majima, a citizen of Japan, of 57 Nishi Aketa-cho, Higashi-Kujo, Minami-ku, hereby declare and state as follows.

1. I graduated from Department of Material and Life Science, Graduate School of Engineering, Osaka University.

2. I have been employed by Arkray, Inc. since 2002.

3. I am engaged in research in the fields of isolating nucleic acids techniques.

4. I am one of the inventors named in the above-identified application, and my family name was changed from Hashiguchi to Majima as a result of marriage.

5. I have read and understood the specification of the above-identified application, and the Office Action dated June 4, 2009.

6. The following experiments were performed by myself to examine the effects of high concentration salt on PCR amplification.

7. Comparative Example (DNA isolation in the absence of high concentration salt)

10 µl of blood was added with 90 µl of the extraction buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 1% Triton X-100, 0.1 M NaCl) and suspended for 3 seconds, and the suspension was heated at 98 °C for 5 minutes. During the heating, a gel filtration spin column was prepared. The gel filtration spin column was prepared by filling 600 µl of gel filtration resins (CHROMA SPIN-1000 (CLONTECH)) into the spin column (MicroSpin Empty Column (Amersham Biosciences)), centrifuging the spin column at 700 g for 2 minutes to remove a liquid therein. After heating, the sample was immediately mixed with a vortex mixer. Then, the sample was centrifuged at 500 G for 10 seconds, and 50 µl of the resulting supernatant was supplied to the gel filtration spin column prepared as described above. The spin column was centrifuged at 300 g for 1 minute, thereby eluted a DNA extract solution. The time spent for the isolation was 10 minutes.

8. Example (DNA isolation in the presence of high concentration salt)

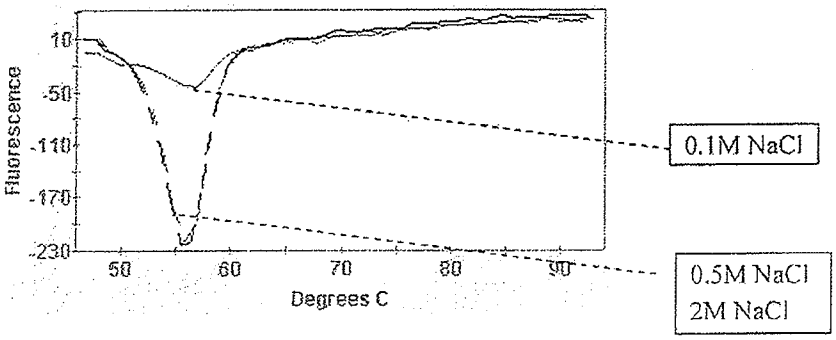
10 µl of blood was added with 90 µl of the extraction buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 1% Triton X-100, 0.5 or 2 M NaCl) and suspended for 3 seconds, and the suspension was heated at 98 °C for 5 minutes. During the heating, a gel filtration spin column was prepared. The gel filtration spin column was prepared by filling 600 µl of gel filtration resins (CHROMA SPIN-1000 (CLONTECH)) into the spin column (MicroSpin Empty Column (Amersham Biosciences)), centrifuging the spin column at 700 g for 2 minutes to remove a liquid therein. After heating, the sample was immediately mixed with a vortex mixer. Then, the sample was centrifuged at 500 G for 10 seconds, and 50 µl of the resulting supernatant was supplied to the gel filtration spin column prepared as described above. The spin column was centrifuged at 300 g for 1 minute, thereby eluted a DNA extract solution. The time spent for the isolation was 10 minutes.

9. PCR reaction

Hundred-fold dilutions (x 100) of the prepared DNA solutions were prepared and used as a template in PCR with Smart Cycler (TAKARA BIO INC.) in which Forward Primer (5'-cacatgtgcaacgcagcg-3') and Reverse Primer (5'- ctcttgccatatgtattggatccc-3') were used as primers to amplify IAPP gene in the presence of a probe (BODIPY FL)-caaagttgttgccggaatgaa-(P) (wherein (P) at the 3' end means being phosphorylated). The reaction solution contained 1 x Gene Taq Buffer (NIPPON GENE CO., LTD.), 1.5 mM $MgCl_2$, 200 µM dNTP mixture, 5 % glycerol, 1 µM of Forward Primer, 0.5 µM of Reverse Primer, and 0.625 U Gene Taq FP (NIPPON GENE CO., LTD.). 1 µl of the template DNA solution was added therein, and the total volume was adjusted to 25 µl by H_2O. The PCR program was as follows. A cycle consisting of heating at 94 °C for 1 second and 66 °C for 18 seconds was repeated 50 cycles. Melting curve analysis (Tm analysis) was performed to confirm whether PCR amplification was successfully performed.

The results of Tm analysis are shown in Table 1 below. In Table 1, the vertical axis represents a primary derivative value of fluorescence intensity with an inverted sign (-dF/dt), and the horizontal axis represents temperature (Celsius).

10. Table 1. Tm analysis for changes in fluorescence intensity



11. As shown in Table 1 above, in the case of 0.1M NaCl (i.e. in the absence of high concentration salt), a blunt peak was observed and thus PCR amplification was not sufficient. On the other hand, in the case of 0.5M or 2M NaCl (i.e. in the presence of high concentration salt), a sharp peak was observed and thus sufficient amplification by PCR was confirmed.

12. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States codes and that such willful, false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: 14, Aug, 2009 By: Satoshi Majima
(Satoshi Majima)